Applied and Environmental Microbiology, Feb. 2008, p. 959–970 Vol. 74, No. 4
0099-2240/08/$08.00+0 doi:10.1128/AEM.01973-07

Effect of Shadowing on Survival of Bacteria under Conditions Simulating the Martian Atmosphere and UV Radiation▽†

Shariff Osman,[1] Zan Peeters,[2] Myron T. La Duc,[1] Rocco Mancinelli,[3] Pascale Ehrenfreund,[2] and Kasthuri Venkateswaran[1]*

Biotechnology and Planetary Protection Group, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California 91109[1]; Leiden Institute of Chemistry, Astrobiology Laboratory, 2300 RA Leiden, The Netherlands[2]; and Carl Sagan Center, SETI Institute, Mountain View, California 94043[3]

Received 28 August 2007/Accepted 4 December 2007

Spacecraft-associated spores and four non-spore-forming bacterial isolates were prepared in Atacama Desert soil suspensions and tested both in solution and in a desiccated state to elucidate the shadowing effect of soil particulates on bacterial survival under simulated Martian atmospheric and UV irradiation conditions. All non-spore-forming cells that were prepared in nutrient-depleted, 0.2-μm-filtered desert soil (DSE) microcosms and desiccated for 75 days on aluminum died, whereas cells prepared similarly in 60-μm-filtered desert soil (DS) microcosms survived such conditions. Among the bacterial cells tested, *Microbacterium schleiferi* and *Arthrobacter* sp. exhibited elevated resistance to 254-nm UV irradiation (low-pressure Hg lamp), and their survival indices were comparable to those of DS- and DSE-associated *Bacillus pumilus* spores. Desiccated DSE-associated spores survived exposure to full Martian UV irradiation (200 to 400 nm) for 5 min and were only slightly affected by Martian atmospheric conditions in the absence of UV irradiation. Although prolonged UV irradiation (5 min to 12 h) killed substantial portions of the spores in DSE microcosms (~5- to 6-log reduction with Martian UV irradiation), dramatic survival of spores was apparent in DS-spore microcosms. The survival of soil-associated wild-type spores under Martian conditions could have repercussions for forward contamination of extraterrestrial environments, especially Mars.

Several environmental factors must be considered when the ability of microbes to survive and proliferate in extraterrestrial environments is assessed (28). It has been postulated that survival and propagation of terrestrial microbes on Mars are possible only if the organisms can withstand extremely low water activity conditions, including complete desiccation (28).

The nutrient-deprived (oligotrophic), ultraclean, and desiccated (constant relative humidity) conditions maintained within spacecraft assembly areas limit the proliferation of microbial life in these environments. Rigorous maintenance procedures, such as regular cleaning (14, 30), HEPA air filtration, and constant humidity and temperature control, make these facilities inhospitable to microbial life. Much like similarly maintained facilities in the medical and pharmaceutical industries (7), these settings have been dubbed "extreme" with respect to microbial persistence (5, 49). Microbes isolated from the clean rooms are often found to be remarkably resilient and capable of tolerating a variety of harsh environmental conditions. A recent report demonstrated that not only spore-forming *Bacillus* species but also a diverse suite of equally "hardy," physiologically flexible nonsporulating bacterial species persist in the inhospitable conditions of clean-room environments (22).

The endospores of several *Bacillus* species isolated from spacecraft assembly facilities have previously exhibited various levels of resistance to H_2O_2 treatment, desiccation, γ-radiation, and UV radiation (19, 24, 48), as well as simulated Martian conditions (32, 45). Furthermore, *Bacillus subtilis* spores have been shown to survive the conditions of low-Earth orbit for up to 6 years (15, 16). However, these spores could survive exposure for 6 years only with shielding from UV radiation (15, 17). The solar UV flux at the Martian surface is considerably less than that in interplanetary space (15, 44), and UV attenuation at the planet's surface is dependent upon ever-changing atmospheric conditions (3, 12, 29).

On Mars, windblown dust has been hypothesized to attenuate solar UV irradiation, resulting in partial or complete shielding of viable bacteria (10, 13, 17, 27, 36, 44). These UV-shielding effects have not been limited to specific soil types or particle sizes. Simulated Martian dust (17, 27), Fe-montmorillonite (27), limonite (10, 13), crushed red sandstone (17), and terrestrial field soils (17, 36) have all been shown to effectively protect bacterial spores. When these reports were reviewed (44), the consensus was that covering *B. subtilis* spores with dust particles that were 2 to 8 μm in diameter resulted in total inactivation of spores after 1 h of UV exposure, while it took 8 h of exposure to UV irradiation to inactivate spores blanketed in dust particles that were up to 50 μm in diameter. Only a 0.5-mm contiguous layer of simulated Martian dust was able to protect *B. subtilis* from longer exposure to normal Martian UV flux (44). None of these studies tested the survival of vegetative bacterial cells under simulated Martian conditions.

The findings reported here are the findings of the first examination of the survival of non-spore-forming bacteria under

* Corresponding author. Mailing address: Biotechnology and Planetary Protection Group, Jet Propulsion Laboratory, California Institute of Technology, Mail Stop 89, 4800 Oak Grove Dr., Pasadena, CA 91109. Phone: (818) 393-7245. Fax: (818) 393-4176. E-mail: kjvenkat@jpl.nasa.gov.

† Supplemental material for this article may be found at http://aem.asm.org/.

▽ Published ahead of print on 14 December 2007.

TABLE 1. Qualitative analysis of the UV tolerance of several extremotolerant bacteria isolated from spacecraft assembly facilities

Bacteria	Strain[a]	GenBank accession no.	Growth after exposure of cultures to UVC (254 nm)[b]			
			No UV	200 J m^{-2}	500 J m^{-2}	1,000 J m^{-2}
Non-spore-forming bacteria						
Arthrobacter sp.	KSC_Ak2i	DQ870702	+	+	+	+
Brachybacterium paraconglomeratum	LMA_AkL5	DQ870709	+	+	+	−
Kocuria rosea	KSC_Ak6a	DQ870700	+	+	−	−
Microbacterium arborescens	KSC_Ak2e	DQ870704	+	+	+	+
Microbacterium aurum	JSC_Ak7–2	DQ870743	+	+	−	−
Microbacterium schleiferi	LMA_AkK1	DQ870710	+	+	+	+
Micrococcus mucilaginosus	KSC_Ak4f	DQ870701	+	+	−	−
Staphylococcus epidermidis	JSC_Ak8–2	DQ870740	+	+	+	−
Spore-forming vegetative cells						
Bacillus pumilus	JSC_Ak9–3		+	+	−	−
Bacillus pumilus	JSC_Ak10–3		+	+	−	−
Bacillus sp.	JSC_Ak7–1	DQ870739	+	+	−	−
Exiguobacterium acetylicum	KSC_Ak2f	DQ870703	+	+	+	+
Oceanobacillus sp.	JPL_Ak1	DQ870753	+	−	−	−
Gram-negative bacteria						
Brevundimonas diminuta	KSC_Ak3a	EF191247	+	+	+	+
Pseudomonas stutzeri	KSC_Ak10c	DQ870705	+	+	−	−
Sphingomonas oligophenolica	JSC_Ak7–4	DQ870741	+	−	−	−
Sphingomonas trueperi	JSC_Ak7–3	DQ870742	+	+	+	+
Spores (reference strain)						
Bacillus pumilus	SAFR-032	AY167879	+	+	+	+

[a] JPL, Jet Propulsion Lab; LMA, Lockheed Martin Aeronautics; JSC, Johnson Space Center; KSC, Kennedy Space Center.
[b] +, growth after exposure; −, no growth.

simulated Martian conditions. Also tested was the hypothesis that conditions resulting in a reduced cellular water content (e.g., vacuum and extreme desiccation) are major predisposing factors that would select for both microbial persistence in interplanetary space and subsequent proliferation on the Martian surface. A low-nutrient Atacama Desert soil sample was used to simulate Martian aeolian dust in an attempt to elucidate any effects of shielding from UV radiation, as measured by survival indices for bacterial cells and spores following exposure. Such information should prove to be invaluable for future risk assessment of forward contamination (41) and should increase our general understanding of the potential for microbial survival and proliferation on the surface of or within the subsurface of Mars.

MATERIALS AND METHODS

Isolation and identification of microbes from spacecraft and associated environments. *Bacillus pumilus* SAFR-032 was isolated from the Jet Propulsion Laboratory spacecraft assembly facility (19), and other bacterial strains were retrieved from various assembly facilities as described previously (22). With the exception of SAFR-032, all bacteria exhibited tolerance to high pH (pH 11.0). Bacterial small-subunit rRNA genes of purified strains were PCR amplified with the eubacterially biased B27F and B1492R primers, and PCR conditions described elsewhere were used (40). After purification with QIAquick columns (Qiagen), 16S rRNA gene amplicons were fully, bidirectionally sequenced. The identities and phylogenetic relationships of organisms examined in this study were determined by comparison of individual 16S rRNA gene sequences to sequences in the public database (http://www.ncbi.nlm.nih.gov). Evolutionary trees were constructed via phylogenetic analysis using parsimony software (http://paup.csit.fsu.edu). The sources of the tested strains along with the GenBank accession numbers for their 16S rRNA gene sequences are shown in Table 1.

Bacterial propagation. All non-spore-forming bacteria were grown in R2B medium (Difco) and incubated at 25°C for 48 h. The resulting cultures were washed twice and finally resuspended in sterile phosphate-buffered saline (PBS) (pH 7.2; BBL) to remove residual nutrients. Spore-forming bacteria were grown on tryptic soy agar (TSA) and incubated at 32°C for 24 h. A nutrient broth sporulation medium was used to induce sporulation, and spores were harvested and purified as previously described (33, 42). Briefly, a single purified colony was inoculated into nutrient broth sporulation medium, and after 3 days of growth at 32°C cultures were examined by microscopy to determine the level of sporulation. Once the percentage of free spores in a culture was ~99%, the culture was harvested and spores were purified. Purified spores were resuspended in sterile deionized water, heat shocked (80°C for 15 min), and stored at 4°C in glass tubes.

Selection of UV_{254}-resistant bacteria. Purified spores of *B. pumilus* SAFR-032 and vegetative cells of several non-spore-forming bacteria were diluted in PBS (pH 7.2) to obtain a density of 10^6 or 10^7 bacteria per ml. The initial bacterial density was estimated by serial dilution plating before each exposure. A low-pressure handheld mercury arc UV lamp (model UVG-11; 254-nm UVC [UV_{254}]; UVP, Inc.) was placed at a fixed height over the sample, and the UV flux at the surface of the spore suspension was measured using a UVX digital radiometer (UVP, Inc.). The exposure times necessary to obtain 200, 500, and 1,000 J m^{-2} of energy at the sample surface were determined (the UV dose rate was 1 J m^{-2} s^{-1}). Each bacterial suspension was placed in a biohood in an uncovered 50-mm glass petri dish and stirred with a magnetic stir bar while it was exposed to UV irradiation under aseptic conditions. Strains surviving 1,000-J m^{-2} irradiation were selected for quantitative lethal dose curve analysis. Samples (100 μl) of these strains were removed at specific time points, serially diluted, and spread on TSA or R2A medium plates. Bacteria for which the dose at which 90% of the spores or cells were inactivated remained >200 J m^{-2} after exposure to a minimum of 200 J m^{-2} were chosen for further experiments.

Atacama Desert soil characterization. The Atacama Desert is among the driest places on Earth. A soil sample was collected from the Arequipa region in Peru (a northern extension of the Atacama Desert) for use as a matrix material for Martian simulation experiments. This soil sample was obtained from the top of a small slope (16°44′34.0″S, 72°02′36.7″W) at an altitude of 1,165 m. The extremely arid core of the Atacama Desert was previously selected as a model for Mars because of its low moisture content (31) and low nutrient content, as described below. The pH of the soil sample was 4.0, and the initial redox potential was 145 mV; the redox potential decreased slowly over time to a final value of 8 mV after removal of dissolved oxygen (Z. Peeters, R. Quinn, Z. Martins, L. Becker, P. Willis, F. Grunthaner, and P. Ehrenfreund, submitted for publication). The main chemical constituents in the soil were calcium and sulfate.

The sample also contained low but measurable levels of organic materials, and L-amino acids and glycine were the most abundant amino acids. Furthermore, four Martian diurnal cycle simulations with Arequipa soil after the soil sample was spiked with D-alanine were performed (Peeters et al., submitted). Strong degradation of D-alanine (~ 40%) was measured, which was indicative of highly reactive soil mineralogy.

Preparation of microcosms using Atacama Desert soil. One gram of Atacama Desert soil was suspended in 100 ml of sterile, molecular-grade Sigma water (Sigma, St. Louis, MO) and stirred thoroughly for 1 min. After settling, the resulting supernatant was pour plated (R2A medium) and incubated at 25°C for 14 days to enumerate the cultivable bacteria. In addition, a bead beating step was employed before a multisolvent extraction method (18) was used to isolate DNA from 10 ml of the soil suspension. Culture-based analysis did not reveal the presence of any bacterial colonies after 14 days of incubation, and DNA extracts from the soil suspensions did not yield any PCR-amplifiable 1.5-kb 16S rRNA gene fragments (23). To prepare desert soil and a desert soil extract, 2 g of Atacama Desert soil was aseptically weighed and suspended in 2 liters of autoclaved, nanopure water (Millipore, Milford, MA), which was then stirred for 15 min. After large particulates were allowed to settle (2 h), the supernatant was carefully removed and aseptically filtered through sterile 60-μm filters. The resulting filtrate was designated desert soil (DS). The 60-μm-filtered DS suspension was then split in half, and one half (1 liter) was subjected to filtration with 0.2-μm filters and was designated desert soil extract (DSE). The sterility of the DS and DSE solutions was evaluated by culturing in R2A medium and attempted extraction of DNA. As was the case previously, cultivable bacteria were not observed on R2A medium plates, nor were 16S rRNA genes amplified from either solution. The DS and DSE suspensions were divided into 100-ml aliquots before they were inoculated with spores or vegetative cells. In a similar manner, bacterial suspensions in sterile PBS or spore suspensions in sterile Sigma water were prepared without desert soil for comparative analyses.

UV_{254} resistance of Atacama Desert microcosms. The DS and DSE microcosms inoculated with spores and cells mentioned above were exposed to UVC (254 nm) in solution. As described above, bacterial suspensions were placed in uncovered 50-mm glass petri dishes containing a magnetic stir bar and exposed to UV irradiation under sterile conditions. Strains surviving cumulative doses (200, 500, and 1,000 J m^{-2} irradiation) were enumerated by spread plating on TSA or R2A medium.

Spacecraft-qualified aluminum coupon preparation. High-grade aluminum (Al 6061-T6) currently being considered for use in in situ and/or sample return hardware was selected for this study, based on the recommendations of Jet Propulsion Laboratory Spacecraft Assembly Engineering Group. Mill-finished aluminum (15 roughness measurement system; 0.65% Si, 0.44% Fe, 0.27% Cu, 0.02% Mn, 0.96% Mg, 0.20% Cr, 0.02% Ti) was cut into "coupons" that were 1 by 2.5 cm. Care was taken to avoid scratching on the material's surface, and coupons were visibly inspected and were rejected if they were scratched or did not have clean edges.

All coupons were precleaned with clean-room-grade polyester wipes (Coventry 6209 c-prime, Freon washed), saturated with acetone to remove residual adhesive, degreased with Freon vapor for 1 h, and finally rinsed with isopropyl alcohol and dried in filtered air at room temperature. Next, coupons were arranged in a single layer on a quartz plate, placed in a UVC CL-1000 crosslinker, and surface sterilized for 20 min. The sterility of the metal coupons was randomly assayed by placing UV-sterilized coupons into tryptic soy broth (TSB) and incubating them at 25°C for vegetative bacteria and at 32°C for *Bacillus* spores.

Bacterial seeding, desiccation, and recovery. The concentration of bacteria in each initial microcosm was determined by serial dilution spread plate assays. Each precleaned, sterile coupon was seeded with 100 μl of a spore or cell suspension calculated to contain ~10^6 total spores or ~10^5 to 10^7 cells. The aluminum coupons seeded with bacteria were dried at room temperature overnight and subsequently stored in sterile screw-cap 15-ml Falcon centrifuge tubes. Following exposure to simulated Martian conditions, seeded coupons were placed in sterile 15-ml tubes, suspended in 5 ml PBS, and vortexed for 1 min at 3,200 rpm. Bacterial survival was assayed via spread and pour plate methods. Processed coupons were finally placed into TSB to confirm the presence or absence of any viable, cultivable microbes still adhering to the aluminum. Some coupons were not inoculated with bacteria or spores and served as controls for comparative analysis.

Martian atmospheric model. The solar simulator employed in this study (Sciencetech model SF150 with an AM0 filter) provided a beam spot that could accommodate seven samples per experiment. The output of the lamp was measured at the sample site using a UV sensor (Solartech model 8.0) with a known sensitivity profile between 240 and 280 nm (peak, 257 nm). The integrated intensity measured by the UV sensor was used to scale the irradiance spectrum shown in Fig. 1. The spectrum of the lamp approximated the noon solar spectrum on Mars for equatorial regions and a dust-free atmosphere as modeled previously (37). Integrated over the wavelength range from 200 to 400 nm, the solar simulator had an output of 30 W m^{-2}, compared to 32 W m^{-2} for the Martian model. A full-range light meter (Extech EA30) was used to confirm the stability of the lamp's output for the duration of the experiment.

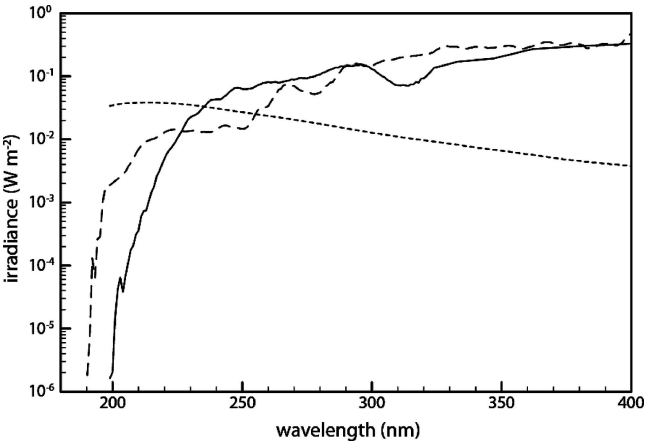

FIG. 1. UV spectra (200 to 400 nm) of the solar simulator (solid line) and the deuterium lamp (dotted line) employed in this study. The lighting spectrum of Mars modeled for near-equator regions at noon and in a dust-free atmosphere by Patel et al. (37) is indicated by the dashed line. The output of the solar simulator was measured and adjusted to match the spectrum of the Mars model. The integrated irradiance over the wavelength range from 200 to 400 nm was 30 W m^{-2} for the solar simulator, 32 W m^{-2} for the Mars model, and 3.4 W m^{-2} for the deuterium lamp.

Deuterium lamp model. A deuterium lamp (Heraeus-Noblelight DX202) was also used to irradiate samples. These samples were irradiated for 12 h under ambient conditions in air. The output of the deuterium lamp was measured with the Sciencetech UV sensor at the level of the samples. Integrated over the wavelength range from 200 to 400 nm, the deuterium lamp had an output of 3.4 W m^{-2}. The spectrum of the deuterium lamp is shown in Fig. 1.

Martian simulation experiment. Samples were irradiated for 5 min, 30 min, or 12 h with a solar simulator under ambient conditions in air. These samples, which were exposed to only Martian UV radiation, were designated MUV. The Mars simulation chamber that was used to expose samples to the Martian atmospheric conditions has been described elsewhere (Peeters et al., submitted). A brass block connected to copper cryogen feed pipes was used as a cooling stage. The top of the brass block was fitted with a highly polished flat copper plate to optimize the thermal conduction between the cooling stage and the aluminum coupons holding the samples. The cooling stage was placed in a small vacuum chamber with a background pressure of ~10^{-4} Pa. A small valve allowed introduction of CO_2 (99.996%; Praxair) into the vacuum chamber. Above the cooling stage a UV-grade quartz window was mounted, which allowed admittance of light having wavelengths down to 200 nm into the Mars simulation chamber. For the Mars simulation experiments the samples were irradiated with the solar simulator. The output of the lamp as measured by the UV sensor was the same as the output used for the irradiation experiments outside the Mars simulation chamber.

The experimental setup was prepared by placing samples on the cooling stage in the Mars simulation chamber. After the lid was closed, the pressure in the chamber was adjusted to 10^3 Pa with a pump. The chamber was then filled with CO_2 at a pressure of 10^5 Pa. This procedure was repeated three times to replace (dilute) the air in the chamber with a CO_2 atmosphere, while a pressure that did not drop below the average Martian pressure (7×10^2 Pa) was maintained. The experiments started when the pressure in the chamber reached 7.0×10^2 Pa. In the Mars simulation experiments, samples were exposed to solar simulator radiation for 5 or 30 min at room temperature (MUVP samples); to 12 h of solar simulator radiation at room temperature, followed by incubation for 12 h at −60°C without radiation (MUVP-1440 samples); or to 12 h without irradiation at room temperature, followed by 12 h of incubation at −60°C (MUVM samples). All experiments were performed with 7×10^2 Pa CO_2.

Microscopy. An Olympus (Nepa, CA) phase-contrast microscope (BX-60) was used to determine the refractile nature of the spores. A field emission environmental scanning electron microscope (FE-SEM) (Philips XL30; FEI Co., Potomac, MD) was used for nondestructive examination of spores and vegetative cells (see below). Specimen preparation procedures, which often lead to sample artifacts, are not necessary when the FE-SEM is used. In addition, a transmission electron microscope was used to examine the surface details and cross-sections of the spores and cells, respectively, by established methods (4). Briefly, the spores or cells were suspended in an equal volume of 5% glutaraldehyde in aqueous 100 mM HEPES buffer (pH 6.8) containing 2 mM $MgCl_2$ as a fixative. After several washes (three washes ranging from 10 min to overnight incubation) in the HEPES buffer fixative solution, spores or cells were allowed to sit for 2 to 4 h at room temperature. The spore or cell pellets were then suspended in 1% osmium tetroxide for 2 to 4 h at room temperature and washed with the HEPES buffer. The fixed spores or cells were embedded in agar, and the agar cubes were dehydrated using successive ethanol treatments (70 to 100% ethanol). The embedding and thin-sectioning procedures used have been described elsewhere (4).

An FE-SEM provides high spatial resolution combined with low electron beam accelerating voltage. The low beam voltage of the FE-SEM allows examination of electrical insulators without deposition of a surface-conducting (carbon or metal) layer to eliminate specimen charging, which can lead to a distorted and often completely unusable image. The deposition of a conducting material to control charging can complicate analysis of the results. In many situations, a low electron beam voltage intrinsically results in a much sharper image, especially for thin structures composed of elements with low atomic numbers. A Phillips (Hillsboro, OR) FE-SEM (XL-50) was used to analyze the majority of the samples. Elemental analysis can be performed with an SEM equipped with an energy-dispersive X-ray (EDX) analyzer. EDX analysis is based on analysis of the characteristic X rays emitted when an electron beam is incident on a sample. Unfortunately, the spatial resolution obtainable with EDX analysis is at best about 1 μm. The acceleration voltage used to analyze aluminum samples was ~10 to 20 kV. In the high-vacuum mode secondary electron images were acquired for both metals. Similar settings were maintained when different models or SEM instruments were used.

RESULTS

Identification of UV irradiation-resistant microbes. Of 17 alkalotolerant (pH 11.0) species identified, 13 (~76%) were gram positive and four strains were identified as the members of the *Alpha-* and *Gammaproteobacteria* (Table 1). The actinobacterial alkalotolerant isolates belonged to the genera *Arthrobacter*, *Brachybacterium*, *Kocuria*, *Microbacterium*, and *Micrococcus*. The alkalotolerant spore-forming rods were species of *Bacillus*, *Oceanobacillus*, and *Exiguobacterium.* Three alkalotolerant isolates represented novel bacterial species, based on 16S rRNA gene sequence dissimilarity, and were most closely related to species of *Arthrobacter* (KSC_Ak2i), *Bacillus* (JSC_Ak7-1), and *Oceanobacillus* (JPL_Ak1).

Survival of hydrated bacteria under UV_{254} radiation. Of the 12 non-spore-forming strains that exhibited UVC resistance, 4 spacecraft-associated isolates were chosen for further study based on their enhanced survivability (elevated doses at which 90% of the spores or cells were inactivated) (data not shown). These isolates included two actinobacteria (*Arthrobacter* sp. strain KSC_Ak2i and *Microbacterium schleiferi* LMA-AkK1) and two gram-negative species (*Brevundimonas diminuta* KSC_Ak3a and *Sphingomonas trueperi* JSC_Ak7-3). In addition to these four strains, *B. pumilus* SAFR-032 was selected as a reference control since this strain has been used in numerous previous resistance studies (19, 25, 32). Survival indices resulting from exposure of PBS-hydrated spores or cells to UVC (254 nm) irradiation demonstrated, as expected, that spores were much more resistant to UVC (less than a 1-log reduction) than any of the vegetative cells tested were (see Fig. S1 in the supplemental material). Vegetative cells of two of the non-spore-forming species, *S. trueperi* and *B. diminuta*, showed ~6-log reductions when they were exposed to 200 J m^{-2} of UVC irradiation. *M. schleiferi* LMA_AkK1 and *Arthrobacter* sp. strain KSC_Ak2i exhibited greater resistance, showing only ~1- and ~3-log reductions, respectively. When spore- and vegetative cell-DSE microcosms were exposed to UVC, a declining trend in microbial viability was observed for all preparations tested, including spores (Fig. 2A). For example, SAFR-032 spores showed a 2-log reduction in UVC-irradiated DSE microcosms (cumulative dose, 1,000 J m^{-2}) (Fig. 2A), compared to the negligible reductions observed under similar UV-irradiated conditions for PBS-spore microcosms (see Fig. S1 in the supplemental material). Similarly, both *M. schleiferi* LMA_AkK1 and *Arthrobacter* sp. strain KSC_Ak2i showed at least a 1-log-greater reduction in DSE microcosms. The comparatively low salt content of the DSE microcosms compared to the PBS microcosms could potentially have influenced the recovery of UV-damaged cells. In contrast to the DSE microcosms, the generally larger soil particles (<60 μm) in the DS microcosms appeared to have shielded both spores and cells from the biocidal effects of UV radiation (Fig. 2B). Continuous stirring with sterile magnetic beads was not adequate to eliminate the shadowing effect in the DS suspensions. Furthermore, the effect of the shadowing was even more pronounced in DS microcosms containing *S. trueperi* and *B. diminuta*, both of which yielded surviving cells following exposure to 1,000 J m^{-2} UV, whereas in both the PBS and DSE microcosm counterparts there were no survivors after exposure to 500 J m^{-2} or a higher dose.

Atacama Desert soil characterization. Figure 3 shows electron micrographs of desiccated DSE (Fig. 3A) and DS (Fig. 3B) microcosms. When dissolved salts were dehydrated during desiccation, uniform crystals formed and were evident in DSE microcosms (Fig. 3A, inset). However, these crystals did not form aggregated clumps but rather dissociated from each other during desiccation. When spores (Fig. 3C) and cells (Fig. 3E, 4A, 4C, and 4E) were mixed with DSE, uniform monolayers were observed. Clumps and/or aggregates of spores or cells were seldom noticed. However, on the aluminum coupons that were seeded with DS microcosms and left to desiccate there were clumps of soil particles harboring spores and cells (Fig. 3 and 4). Even though 50% of an aluminum coupon was not covered with soil particles, spores and cells tended to associate with such particles, possibly due to the aggregation of biological materials with solids during desiccation.

Influence of desiccation on the survival of spores or cells. Ten percent of cells of *M. schleiferi* and *S. trueperi* survived after 15 days of desiccation in DSE microcosms, while less than 0.2% survival was observed for *Arthrobacter* sp. and *B. diminuta* (see Table S1 in the supplemental material). Invariably, all non-spore-forming cells died after prolonged desiccation (75 days) in nutrient-depleted DSE microcosms. As expected, ~10% (75 days) to 20% (15 days) survival was observed for *B. pumilus* SAFR-032 spores when they were desiccated in DSE microcosms. Vegetative cells generally exhibited similar levels of survival in both DS and DSE microcosms following 15 days of desiccation. However, particulates present in the Atacama Desert soil (DS) enhanced the survival of all spores or cells tested following 75 days of desiccation. Unlike DSE microcosms, with the exception of

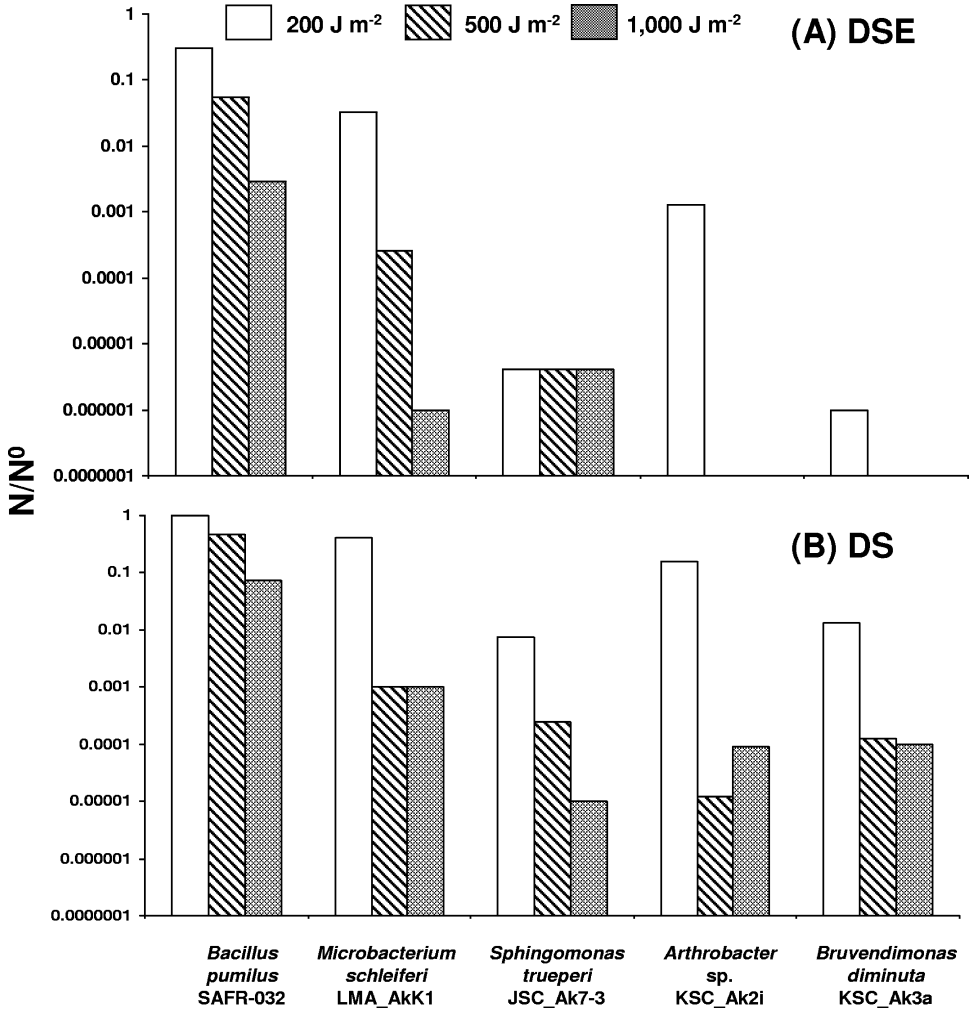

FIG. 2. Survival of hydrated spores or cells in (A) DSE and (B) DS after several cumulative doses of UV_{254} irradiation. N and N^0 represent cfu of the samples without and with radiation, respectively.

B. diminuta, non-spore-forming cells exhibited only 4- to 5-log reductions when DS microcosms were desiccated for 75 days. The DS-*B. diminuta* microcosm contained no viable cells after 75 days of desiccation. With DS-spore microcosms, desiccation had no effect after 15 days (100% survival), and ~10% of the spores could be retrieved after 75 days of desiccation, as observed for DSE microcosms.

Survival of desiccated spores or cells under UV_{254} radiation. The results for UV_{254} irradiation of desiccated DSE microcosms on aluminum are shown in Fig. 5A. As expected, spores displayed enhanced resistance to UVC compared to most of the vegetative cells tested. However, among the non-spore-forming bacteria, *M. schleiferi* LMA_AkK1 and *Arthrobacter* sp. strain KSC_Ak2i cells exhibited unusually high resistance to UVC when they were mixed with DSE. As observed with spores, for *Arthrobacter* sp. seeded in DSE there was only a 3-log reduction in viability after exposure to 1,000 J m^{-2} UVC (Fig. 5A). The resistance observed for these non-spore-forming bacteria when they were in DSE and PBS microcosms was greater than that of vegetative cells of *B. pumilus* SAFR-032 (data not shown). Conversely, *S. trueperi* and *B. diminuta* showed ~4- to 5-log reductions after exposure to 200 J m^{-2} UVC irradiation. The difference between the two sets of bacteria may be a consequence the extremely high tolerance of *Microbacterium* and *Arthrobacter* species to desiccation (20).

The effect of the presence of DS on the viability of spores and cells in desiccating conditions is shown in Fig. 5B. Invariably, all DS microcosms dried onto aluminum coupons showed higher viability even after exposure to 1,000 J m^{-2} UVC, except for the *B. diminuta* cells, which proved to be noncultivable even after an exposed coupon was incubated in liquid medium. Spores in DS microcosms exhibited only a 1-log reduction, and all bacterial cells tested showed ~2- to 4-log-greater survival in DS desiccated microcosms than in DSE desiccated microcosms. However, the lethal effect of UVC was still apparent, as the *B. diminuta* desiccated cells mixed with DS were all killed after exposure to 1,000 J m^{-2} UVC irradiation. The phenomenon of increased viability following UVC exposure may be attributed to the shadowing effect of large soil particles and the inability of UVC to make direct contact with and lethally affect all of the bacteria tested. The higher survival

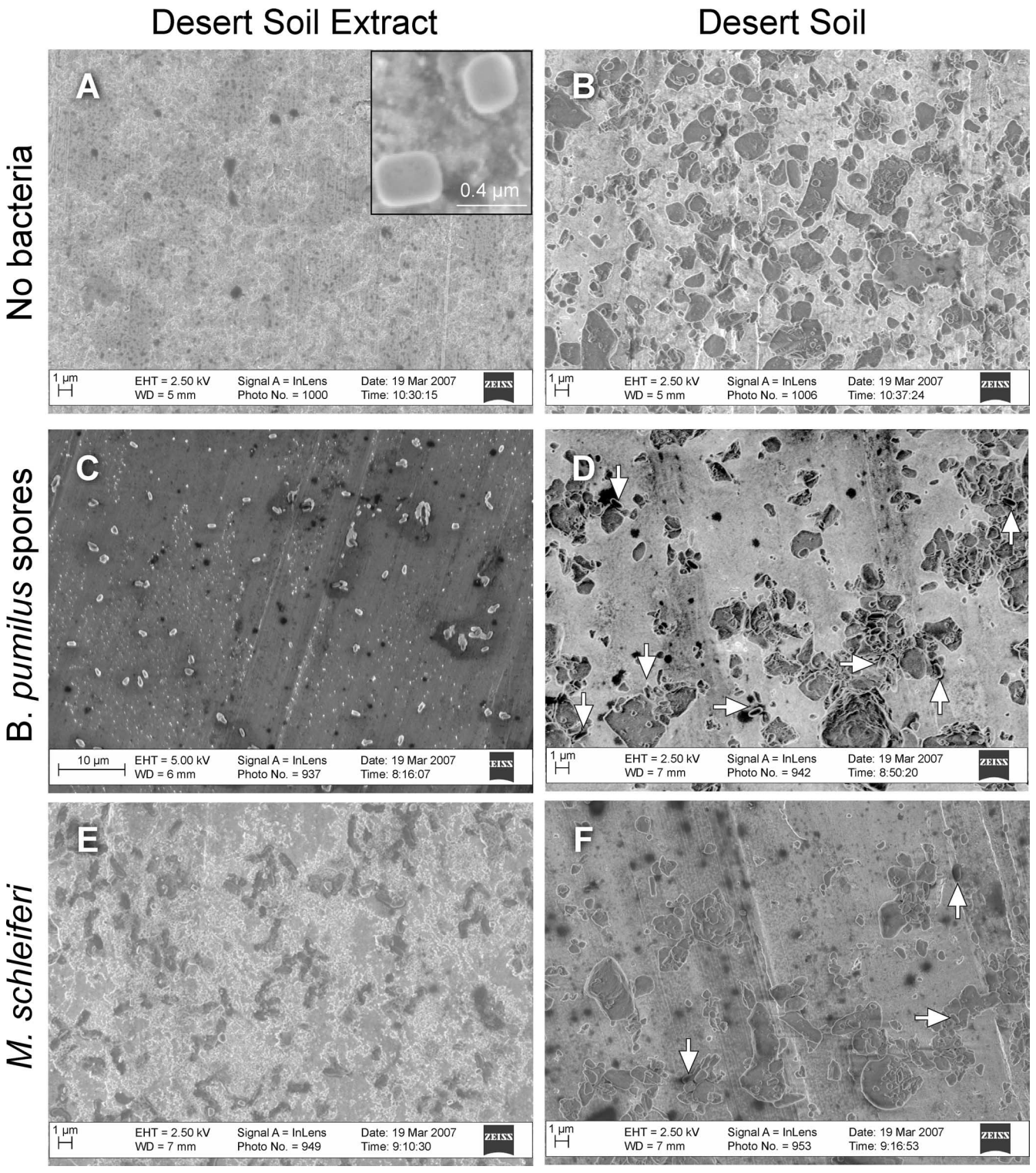

FIG. 3. Environmental scanning electron micrographs of several bacteria and spores prepared in DSE and DS and desiccated on spacecraft-qualified aluminum 6061. The arrows indicate where spores or bacterial cells are present. Some spores or bacterial cells were exposed to the UV, and some were hidden under soil particles.

indices observed for the *Sphingomonas* and *Bruvendimonas* cells inoculated into DS than for the *Sphingomonas* and *Bruvendimonas* cells inoculated into DSE were expected. Higher mineral and trace element concentrations, not to mention the much larger particles, present in the soil may have bolstered the survival of these cells, which otherwise would have been susceptible to desiccation-induced death. In addition, the retention of a soil moisture content higher than that of the DSE

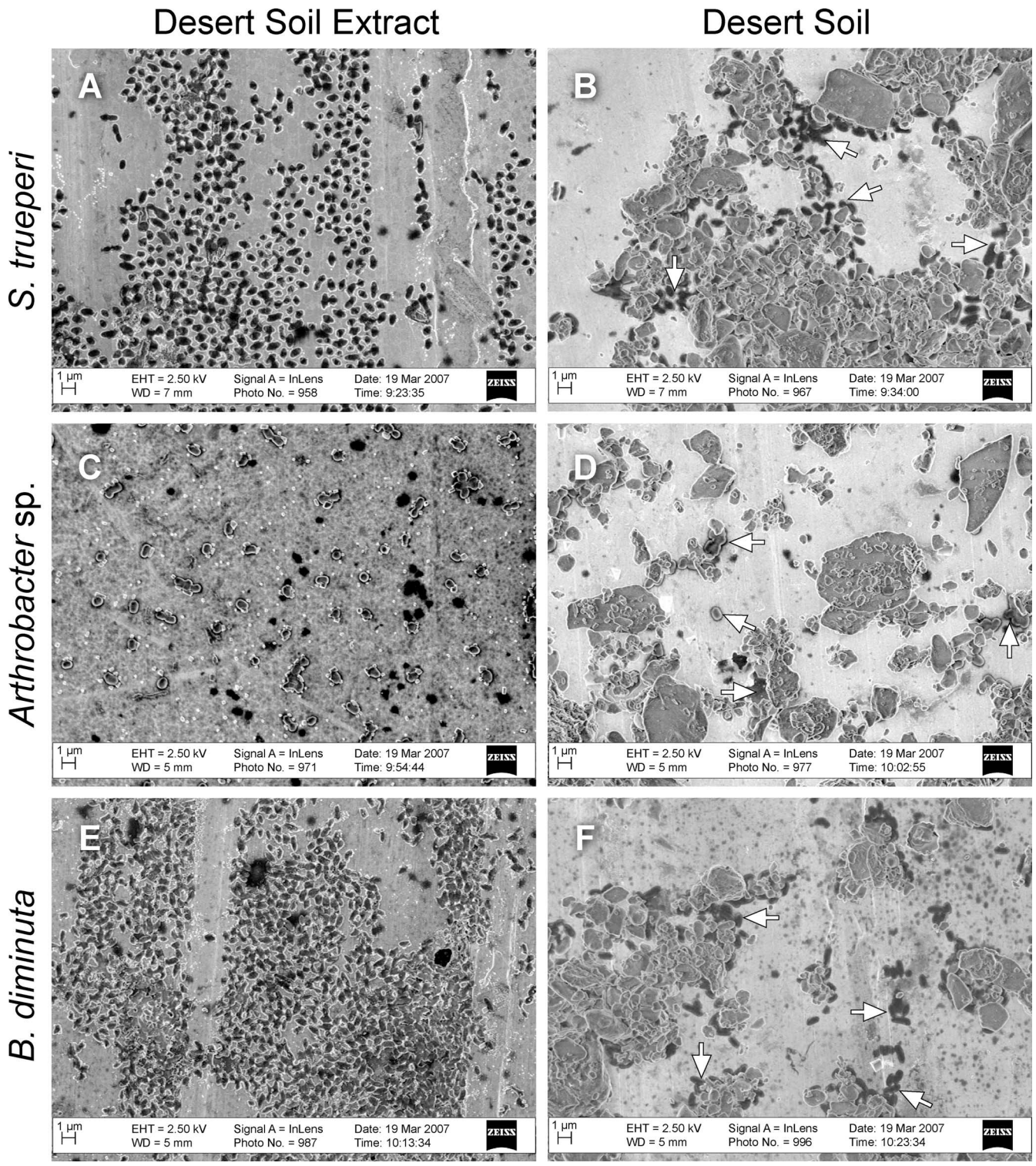

FIG. 4. Environmental scanning electron micrographs of several bacteria and spores prepared in DSE and DS and desiccated on spacecraft-qualified aluminum 6061. The arrows indicate where bacterial cells are present. Some cells were exposed to the UV irradiation, and some were hidden under soil particles.

microcosms may also have bolstered the survival of these cells when they were DS associated.

Survival of desiccated spores or cells following deuterium lamp radiation. Integrated over the wavelength range from 200 to 400 nm, the deuterium lamp had an output of 3.4 W m^{-2}. None of the desiccated DSE or DS microcosms containing non-spore-forming cells exhibited growth after 12 h of irradiation with the deuterium lamp (see Table S2 in the sup-

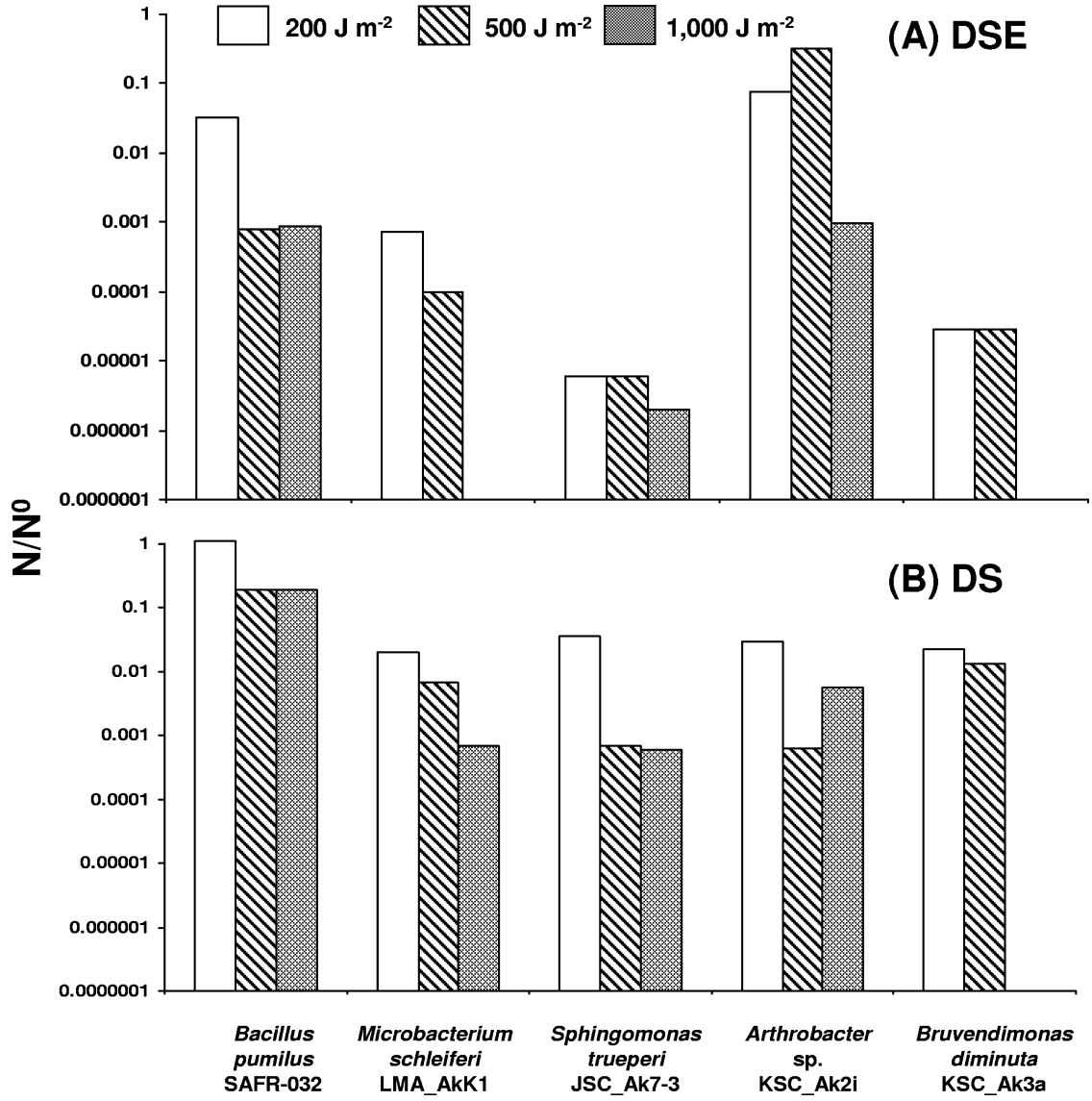

FIG. 5. Survival of desiccated spores or cells on spacecraft-qualified aluminum 6061 after several cumulative doses of UV_{254} irradiation. (A) DSE. (B) DS. N and N^0 represent cfu of the samples without and with radiation, respectively.

plemental material). The sporicidal effects of UV irradiation were observed for the *B. pumilus* spores when DSE-desiccated coupons were exposed to deuterium irradiation for 12 h. However, ~0.2% of the 75-day DS-desiccated spores (initial spore concentration after 75 days of desiccation, 4.3×10^6 per coupon) survived UV irradiation with the deuterium lamp for 12 h.

Survival of spores under simulated Martian conditions. The elimination of non-spore-forming cells from desiccated aluminum coupons by simulated Martian conditions was inconsistent. Although prolonged (75 days) desiccation effectively killed non-spore-forming bacterial cells, in some cases sterility of the aluminum coupons was not observed after exposure for 12 h to Martian UV irradiation (total irradiation, 30 W m^{-2}) and Martian atmospheric pressure (7×10^2 Pa). It is possible that water molecules trapped in the uneven surfaces of the unpolished aluminum material (47) mediated the survival of a subset of bacterial cells. Hence, the lethal effect of simulated Mars UV irradiation and atmospheric conditions was not efficiently documented for the desiccated non-spore-forming cells. Cells of *M. schleiferi* LMA_AkK1 were consistently recovered from desiccated coupons (both DSE and DS microcosms) after exposure to Martian atmospheric conditions without UV (MUVM) both following incubation for 12 h at −60°C and following incubation for 12 h at room temperature (25°C). As observed in other experiments, desiccated DS microcosms containing *M. schleiferi* exhibited at least 1-log-higher survival than their desiccated DSE counterparts (see Table S2 in the supplemental material).

In general and as expected, spores survived under simulated Martian conditions. Figure 6A shows the effect of simulated Martian conditions on spores desiccated in DSE microcosms. Simulated atmospheric conditions (8) without UV had a minimal effect on the survival of spores (less than a 1-log reduc-

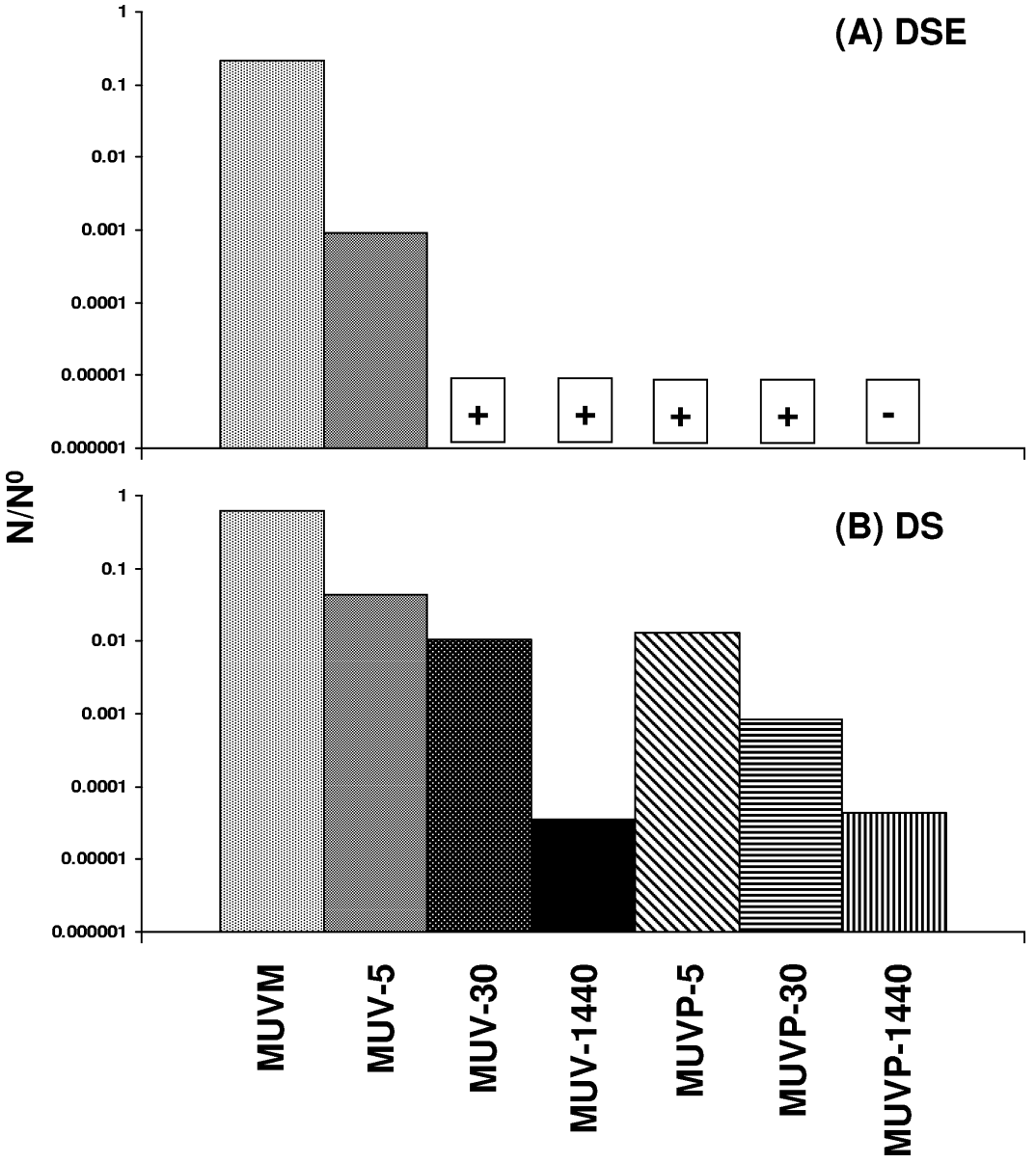

FIG. 6. Survival of desiccated *B. pumilus* SAFR-032 spores on spacecraft-qualified aluminum 6061 under various simulated Mars conditions. MUVM, incubation in the Mars atmosphere without UV irradiation for 12 h at room temperature, followed by 12 h at −60°C; MUV-5, incubation with Mars UV irradiation in air for 5 min; MUV-30, incubation with Mars UV irradiation in air for 30 min; MUV-1440, incubation with Mars UV irradiation in air for 12 h; MUVP-5, incubation with Mars UV irradiation in the Mars atmosphere for 5 min; MUVP-30, incubation with Mars UV irradiation in the Mars atmosphere for 30 min; MUVP-1440, incubation in the Mars atmosphere for 12 h with Mars UV irradiation at room temperature, followed by 12 h with no UV irradiation at −60°C; +, not detected as CFU but detected by incubating an exposed coupon in liquid medium; −, not detected on solid medium or in liquid medium. All experiments were performed at room temperature, unless otherwise noted. Mars UV irradiation was provided by the solar simulator, and the Mars atmosphere used contained 7×10^2 Pa CO_2. N and N^0 represent cfu of the samples without and with radiation, respectively.

tion). Exposure to only Martian UV irradiation for 5 min resulted in only a 3-log reduction in the size of the spore population. Recovery of spores was not possible when the exposure to Martian UV irradiation was increased from 5 to 30 min or longer. However, the aluminum coupons were not completely sterile following exposure to only Martian UV irradiation, as *B. pumilus* SAFR-032 was cultured when the UV-irradiated coupons were placed in TSB. Observations were also recorded when Martian UV irradiation was combined with simulated atmospheric conditions (MUVP). More than 5 min of MUVP exposure proved to be 100% lethal, but growth was detected when the same coupons were incubated in liquid medium. Exposure of these DSE microcosms for 12 h to MUVP conditions, however, resulted in complete sterility, as verified by the lack of cultivable bacteria even in broth. The ability of the Atacama Desert soil slurry to protect spores from Martian UV irradiation and atmospheric conditions is shown in Fig. 6B. A dramatic increase in spore survival was observed

in all microcosms even though prolonged UV irradiation killed substantial portions of the spore population. However, under similar environmental conditions and in the absence of DS particles (DSE conditions) (Fig. 6A), exposure to Martian UV irradiation for more than 5 min effectively reduced the sizes of spore populations by 5 to 6 logs. Here, for the first time, we show that recovery of spores from aluminum coupons is possible even after exposure to 12 h of UV irradiation and 24 h of a simulated Martian diurnal atmosphere.

DISCUSSION

The intimate association of bacterial endospores with spacecraft and assembly facility-associated surfaces has been well documented, as has the ability of these well-adapted microorganisms to resist UV-based methods of decontamination. Therefore, it is generally accepted that such endospores would be the most likely survivors of spacecraft disinfection and thus pose the greatest risk of forward contamination (22, 24, 32, 40, 43–45). Of the five bacterial species examined here, *B. pumilus* SAFR-032 spores have repeatedly tolerated UVC irradiation very well, as reported elsewhere (44), and demonstrated the greatest tolerance to simulated Martian UV and atmospheric conditions (Fig. 6B). It is presumed that this microbe's remarkable resilience to severe stress, including heating, drying, and radiation (35), is a direct consequence of the maintenance of an ultralow water content in its spore cores (~10 to 25% of dry weight). By keeping the water content low, these spores minimize their interactions with deleterious electron-scavenging radicals, which result from splitting of water molecules (i.e., oxidative stress) (9). In addition, spores of *B. pumilus* are less susceptible to desiccation and are better preserved under reduced pressure (1×10^2 Pa) than under standard terrestrial atmospheric pressure (see Fig. S2 in the supplemental material). This could be a result of the more intense drying of the spore core due to the vacuum. It may be presumed that the 10 to 100% survival of *B. pumilus* SAFR-032 spores in DS or DSE after 75 days of desiccation at simulated Martian pressure (7×10^2 Pa) (Fig. 6A and 6B) is likely attributable to the lower spore core water content. Lower core water content would correlate with a lower occurrence of generated free radicals and thus less damage to core-housed nucleic acids, a hypothesis warranting further study.

While the effects of water content on stress resistance have been tested using pure cultures of microorganisms, relatively little is known about the extent to which the natural habitats of microbes can bolster or counteract this phenomenon. The enhanced UV resistance seen in *Bacillus* spores, for instance, may be partially due to differences in soil chemistry during sporulation. Nicholson and Law (34) isolated *Bacillus* spores directly from Sonoran Desert soil and found that they were much more UV resistant than spores of the standard laboratory strain, *B. subtilis* 168. However, much of the unusually high resistance was lost upon germination, propagation, and subsequent sporulation in culture. The sequestering of particulate-bound water in soils during and following sporulation may have a synergistic effect on the UV resistance of spores. This could explain the differences in UV resistance observed between wild-type bacterial strains isolated from extremely desiccated desert and clean-room environments and strains recultured in the laboratory in liquid media.

While spore hardiness may allow spores to persist in extraterrestrial environments, metabolic dormancy most likely would prevent proliferation upon deposition on the surface of Mars. Furthermore, it has been shown that the vegetative cells of a dosimetry strain, *B. subtilis* 168, were killed at UV_{254} doses of less than 100 J m^{-2} and cells of highly UV-resistant *B. pumilus* strain SAFR-032 were completely eradicated at UV_{254} doses greater than 500 J m^{-2} (32). The finding that non-spore-forming isolates (e.g., *Arthrobacter* sp. and *M. schleiferi*) were capable of surviving such extreme UV_{254} regimens was therefore of great significance. Indeed, *Arthrobacter* sp. and *M. schleiferi* cells were as UV resistant as *B. subtilis* spores in both liquid (32) and desiccated (45) states. It has been reported that the survival indices for multilayer microbes (10^8 cells $sample^{-1}$) following UV irradiation were 4 orders of magnitude greater than those for monolayer *B. subtilis* (10^6 cells $sample^{-1}$) (15). To ensure that the differences in UV resistance observed in these experiments were not due to cell layering effects, vegetative cells were initially screened for UV resistance in a liquid environment (PBS, DSE, and DS). Furthermore, the uniformity of the desiccated cellular and spore monolayers on the surfaces of aluminum coupons was validated by FE-SEM (Fig. 3 and 4).

As a soil dries out, stress due to starvation has a detrimental effect on resident microbes long before the loss of intracellular water becomes a concern (21). *Bacillus* and *Arthrobacter* are two well-known extremely desiccation-tolerant soil-inhabiting genera (20). *Arthrobacter* spp. are arguably the most starvation and desiccation tolerant of all non-spore-forming microbes and are the microorganisms most frequently cultivated from desert soils (20), Antarctic ice (11), the subsurface (46), and clean rooms (22). Early studies demonstrated that *Arthrobacter crystallopoites* can survive extended periods of starvation through slow, sparing catabolism of endogenous substrates (2, 6). It has been shown that, upon desiccation, *A. crystallopoites* exhibited 50% viability after 6 months in air-dried soil, at which time the endogenous catabolism was slowed to a rate at which it could be projected that 50% of cellular carbon would remain after 12 years (1). The desiccation tolerance (~75 days) exhibited by cells of *M. schleiferi* LMA_AkK1 and *Arthrobacter* sp. strain KSC_Ak2i during this study, coupled with their ubiquity in soil and general resilience in the presence of various environmental insults, makes these actinobacteria prime candidates for forward contamination of Mars.

While microbial responses to solute-induced low water activity are relatively well understood (26, 38, 50), studies addressing responses to desiccation-induced low water activities in natural environments remain sparse. It is clear that water limits microbial activity in and on desert soils and rocks (20, 31), yet it is not known how much water is sufficient to trigger the rare, brief periods of microbial proliferation in these environments. We attempted to bridge this knowledge gap by testing the hardiest oligotrophic bacterial cells for tolerance to matrix-induced water stress and thereby establishing a true water threshold for survival in Martian regolith. When cells of *M. schleiferi* LMA_AkK1 (5.1×10^3 cells) that had been desiccated for 75 days were exposed to a simulated Martian atmosphere without UV irradiation for 24 h (12 h at room

temperature, followed by 12 h at −60°C), ~40% of the population survived. This cellular response to low water activity was observed only in the presence of DS, while in DSE microcosms there were no survivors. Furthermore, similar to *B. pumilus* spores, desiccated cells of *M. schleiferi* exhibited survival after 24 h of exposure to a simulated Martian atmosphere (7×10^2 Pa; 12 h at room temperature, followed by 12 h at −60°C). This is the first account of survival of desiccated cells under simulated Mars atmospheric conditions in the absence of protection conferred by rock or salt crystal shielding.

The Martian atmosphere attenuates very little UV light, and calculations have posited that incoming solar radiation would kill the hardiest terrestrial organisms within minutes (29). Thus, the persistence of any life near the surface depends, to a large extent, on how much UV radiation it receives. The results of this study support the interpretations described in previous investigations (32, 44, 45): the survival of spacecraft-borne microbial contaminants would be limited to, at best, a matter of hours on a surface exposed to full sun (Fig. 6A). However, subtle variations in small-scale spacecraft geometry, such as pits, trenches, and overhangs, can have a profound effect on the incident UV fluence rates and may result in "safe havens" for microorganisms and organic molecules. In this study, vegetative cells (not spores) that had been desiccated on aluminum exhibited inconsistent survival following 24 h of exposure to a simulated Martian diurnal atmosphere (see Table S2 in the supplemental material). This could have been a consequence of the uneven surface geometry of the aluminum (47), which may have resulted in small areas where there was UV shielding. Such a reduction in Martian UV flux was modeled to facilitate the persistence of hardy terrestrial microorganisms like *B. pumilis* SAFR-032 for several tens of Martian years (29).

Previous studies on the UV shadowing effect of Martian regolith soils have generated contradictory results (10, 13, 17, 27, 36, 44). The microbial reduction observed in these studies was attributed not to the thickness but rather to the quality of dust layers and the type of UV lamp used (44). As recommended by Schuerger et al. (44), appropriate UV lamps (xenon) and soil particle sizes were selected for this study. The high UVA fluence rates of xenon lamps may not be as lethal to *B. pumilus* SAFR-032 spores (32) as to *B. subtilis* HA101 spores (44). In this study, instead of sprinkling dust on bacterial monolayers (44), cells or spores were mixed with the soil before drying on aluminum coupons. Horneck et al. (17) reported that mixing *B. subtilis* spores directly into fine-grained dusts or soils provided significantly greater protection from UV irradiation than sprinkling dust layers on bacterial monolayers. In these models, it is possible that loosely attached dust particles on top of cells migrate upon application of a space vacuum. Hence, in this study the shielding of microbes from UV irradiation was investigated by mixing cells or spores with organic matter-depleted, 60-μm-filtered Atacama Desert soil. The survival of desiccated DS spores under simulated Martian conditions observed in this study might be attributed to the elevated resistance of the wild-type bacterial spores employed here compared to the spores used in previous studies (44) in which laboratory-attenuated *B. subtilis* HA101 spores were used. Based on this study, it is presumed that the mitigation of UV damage (200 to 400 nm) to dust-covered, desiccated spores and their survival on spacecraft-grade aluminum are strain specific. The more pronounced UV resistance observed for wild-type strains than for laboratory strains and the gradual loss of the resilience of spores allowed to germinate and proliferate in vitro (34) support this hypothesis. Therefore, discussions and conclusions regarding the survival of microbes in extraterrestrial environments based solely on laboratory strains should not be generalized.

ACKNOWLEDGMENTS

Part of this research was carried out at the Jet Propulsion Laboratory, California Institute of Technology, under a contract with the National Aeronautics and Space Administration. This research was funded by an NRA ROSES grant awarded to K.V. Portions of this project were funded by cooperative agreement NNX07AE62A (R.M.) and grant NWO-VI 016.023.003 (P.E. and Z.P.).

We are grateful to members of the Biotechnology and Planetary Protection Group for technical assistance and for collecting the spacecraft-associated microbial strains and to J. Kulleck for the FE-SEM analysis. We thank F. Grunthaner for providing the Atacama Desert sample and J. Rummel for encouragement.

REFERENCES

1. **Boylen, C. W.** 1973. Survival of *Arthrobacter crystallopoietes* during prolonged periods of extreme desiccation. J. Bacteriol. **113:**33–37.
2. **Boylen, C. W., and J. C. Ensign.** 1970. Intracellular substrates for endogenous metabolism during long-term starvation of rod and spherical cells of *Arthrobacter crystallopoietes*. J. Bacteriol. **103:**578–587.
3. **Cockell, C. S., D. C. Catling, W. L. Davis, K. Snook, R. L. Kepner, P. Lee, and C. P. McKay.** 2000. The ultraviolet environment of Mars: biological implications past, present, and future. Icarus **146:**343–359.
4. **Cole, R. M., and T. J. Popkin.** 1981. Electron microscopy, p. 34–51. *In* P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg, and G. B. Phillips (ed.), Manual of methods for general bacteriology. American Society for Microbiology, Washington, DC.
5. **Crawford, R. L.** 2005. Microbial diversity and its relationship to planetary protection. Appl. Environ. Microbiol. **71:**4163–4168.
6. **Ensign, J. C.** 1970. Long-term starvation survival of rod and spherical cells of *Arthrobacter crystallopoietes*. J. Bacteriol. **103:**569–577.
7. **Favero, M. S., J. J. McDade, J. A. Robertsen, R. K. Hoffman, and R. W. Edwards.** 1968. Microbiological sampling of surfaces. J. Appl. Bacteriol. **31:**336–343.
8. **Garry, J. R. C., I. L. T. Kate, Z. Martins, P. Nørnberg, and P. Ehrenfreund.** 2006. Analysis and survival of amino acids in Martian regolith analogues. Meteoritics Planet. Sci. **41:**391–405.
9. **Ghosal, D., M. V. Omelchenko, E. K. Gaidamakova, V. Y. Matrosova, A. Vasilenko, A. Venkateswaran, M. Zhai, H. M. Kostandarithes, H. Brim, K. S. Makarova, L. P. Wackett, J. K. Fredrickson, and M. J. Daly.** 2005. How radiation kills cells: survival of *Deinococcus radiodurans* and *Shewanella oneidensis* under oxidative stress. FEMS Microbiol. Rev. **29:**361–375.
10. **Green, R. H., D. M. Taylor, E. A. Gustan, S. J. Fraser, and R. L. Olson.** 1971. Survival of microorganisms in a simulated Martian environment. Space Life Sci. **3:**12–24.
11. **Gupta, P., G. S. Reddy, D. Delille, and S. Shivaji.** 2004. *Arthrobacter gangotriensis* sp. nov. and *Arthrobacter kerguelensis* sp. nov. from Antarctica. Int. J. Syst. Evol. Microbiol. **54:**2375–2378.
12. **Haberle, R. M., C. P. McKay, J. B. Pollack, O. E. Gwynne, D. H. Atkinson, J. Appelbaum, G. A. Landis, R. W. Zurek, and D. J. Flood.** 1993. Atmospheric effects on the utility of solar power on Mars, p. 845–885. *In* J. S. Lewis, M. S. Matthews, and M. L. Guerrieri (ed.), Resources of near-Earth space. University of Arizona Press, Tucson.
13. **Hagen, C. A., E. J. Hawrylewicz, B. T. Anderson, and M. L. Cephus.** 1970. Effect of ultraviolet on the survival of bacteria airborne in simulated Martian dust clouds. Life Sci. Space Res. **8:**53–58.
14. **Henderson, K.** 2000. Contamination control plan, p. D-19494. JPL, Pasadena, CA.
15. **Horneck, G., H. Bucker, and G. Reitz.** 1994. Long-term survival of bacterial spores in space. Adv. Space Res. **14:**41–45.
16. **Horneck, G., U. Eschweiler, G. Reitz, J. Wehner, R. Willimek, and K. Strauch.** 1995. Biological responses to space: results of the experiment "Exobiological Unit" of ERA on EURECA I. Adv. Space Res. **16:**105–118.
17. **Horneck, G., P. Rettberg, G. Reitz, J. Wehner, U. Eschweiler, K. Strauch, C. Panitz, V. Starke, and C. Baumstark-Khan.** 2001. Protection of bacterial spores in space, a contribution to the discussion on Panspermia. Origins Life Evol. Biosph. **31:**527–547.
18. **Johnson, J. L.** 1981. Genetic characterization, p. 450–472. *In* P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg,

and G. B. Phillips (ed.), Manual of methods for general bacteriology. American Society for Microbiology, Washington, DC.
19. **Kempf, M. J., F. Chen, R. Kern, and K. Venkateswaran.** 2005. Recurrent isolation of hydrogen peroxide-resistant spores of *Bacillus pumilus* from a spacecraft assembly facility. Astrobiology **5:**391–405.
20. **Kieft, T. L.** 2002. Hot desert soil communities, p. 1576–1586. *In* G. Bitton (ed.), Encyclopedia of environmental microbiology. John Wiley, New York, NY.
21. **Kieft, T. L.** 2002. Microbial starvation survival in subsurface environments. John Wiley, New York, NY.
22. **La Duc, M. T., A. E. Dekas, S. Osman, C. Moissl, D. Newcombe, and K. Venkateswaran.** 2007. Isolation and characterization of bacteria capable of tolerating the extreme conditions of clean-room environments. Appl. Environ. Microbiol. **73:**2600–2611.
23. **La Duc, M. T., R. Kern, and K. Venkateswaran.** 2004. Microbial monitoring of spacecraft and associated environments. Microb. Ecol. **47:**150–158.
24. **La Duc, M. T., W. Nicholson, R. Kern, and K. Venkateswaran.** 2003. Microbial characterization of the Mars Odyssey spacecraft and its encapsulation facility. Environ. Microbiol. **5:**977–985.
25. **Link, L., J. Sawyer, K. Venkateswaran, and W. Nicholson.** 2004. Extreme spore UV resistance of *Bacillus pumilus* isolates obtained from an ultraclean spacecraft assembly facility. Microb. Ecol. **47:**159–163.
26. **Mancinelli, R. L., T. F. Fahlen, R. Landheim, and M. R. Klovstad.** 2004. Brines and evaporates: analogs for Martian life. Adv. Space Res. **33:**1244–1246.
27. **Mancinelli, R. L., and M. Klovstad.** 2000. Martian soil and UV radiation: microbial viability assessment on spacecraft surfaces. Planet. Space Sci. **48:** 1093–1097.
28. **MEPAG SR-SAG.** 14 July 2006, posting date. Findings of the Special Regions Science Analysis Group, Mars Exploration Program Analysis Group (MEPAG), NASA, JPL, Pasadena, CA. http://mepag.jpl.nasa.gov/reports/MEPAG_SR-SAG_final1.pdf.
29. **Moores, J. E., P. H. Smith, R. Tanner, A. C. Schuerger, and K. Venkateswaran.** 2007. The shielding effect of small-scale Martian surface geometry on ultraviolet lux. Icarus **192:**417–433.
30. **NASA-KSC.** 1999. Launch Site Requirement Planning Group facilities handbook of Payload Hazardous Servicing Facility (PHSF). Publication K-STSM-14.1.15 rev D. KSC, Cape Canaveral, FL.
31. **Navarro-Gonzalez, R., F. A. Rainey, P. Molina, D. R. Bagaley, B. J. Hollen, J. de la Rosa, A. M. Small, R. C. Quinn, F. J. Grunthaner, L. Caceres, B. Gomez-Silva, and C. P. McKay.** 2003. Mars-like soils in the Atacama Desert, Chile, and the dry limit of microbial life. Science **302:**1018–1021.
32. **Newcombe, D. A., A. C. Schuerger, J. N. Benardini, D. Dickinson, R. Tanner, and K. Venkateswaran.** 2005. Survival of spacecraft-associated microorganisms under simulated Martian UV irradiation. Appl. Environ. Microbiol. **71:**8147–8156.
33. **Nicholson, W., and P. Setlow.** 1990. Sporulation, germination and outgrowth, p. 391–450. *In* C. R. Harwood and S. M. Cutting (ed.), Molecular biological methods for *Bacillus*. John Wiley and Sons, Inc., Hoboken, NJ.
34. **Nicholson, W. L., and J. F. Law.** 1999. Method for purification of bacterial endospores from soils: UV resistance of natural Sonoran desert soil populations of *Bacillus* spp. with reference to *B. subtilis* strain 168. J. Microbiol. Methods **35:**13–21.
35. **Nicholson, W. L., N. Munakata, G. Horneck, H. J. Melosh, and P. Setlow.** 2000. Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. Microbiol. Mol. Biol. Rev. **64:**548–572.
36. **Packer, E., S. Scher, and C. Sagen.** 1963. Biological contamination of Mars. II. Cold and aridity as constraints on the survival of terrestrial microorganisms in simulated Martian environments. Icarus **2:**293–316.
37. **Patel, M. R., J. C. Zarnecki, and D. C. Catling.** 2002. Ultraviolet radiation on the surface of Mars and the Beagle 2 UV sensor. Planet. Space Sci. **50:**915–927.
38. **Potts, M.** 1994. Desiccation tolerance of prokaryotes. Microbiol. Rev. **58:** 755–805.
39. **Puleo, J. R., S. L. Bergstrom, J. T. Peeler, and G. S. Oxborrow.** 1978. Thermal resistance of naturally occurring airborne bacterial spores. Appl. Environ. Microbiol. **36:**473–479.
40. **Ruimy, R., V. Breittmayer, P. Elbaze, B. Lafay, O. Boussemart, M. Gauthier, and R. Christen.** 1994. Phylogenetic analysis and assessment of the genera *Vibrio*, *Photobacterium*, *Aeromonas*, *and Plesiomonas* deduced from small-subunit rRNA sequences. Int. J. Syst. Bacteriol. **44:**416–426.
41. **Rummel, J. D.** 1989. Planetary protection policy overview and application to future missions. Adv. Space Res. **9:**181–184.
42. **Schaeffer, P., J. Millet, and J. P. Aubert.** 1965. Catabolic repression of bacterial sporulation. Proc. Natl. Acad. Sci. USA **54:**704–711.
43. **Schuerger, A. C.** 2004. Microbial ecology of the surface exploration of Mars with human operated vehicles, p. 363–386. *In* C. S. Cockell (ed.), Martian expedition planning. Univelt Publishers, Escondido, CA.
44. **Schuerger, A. C., R. L. Mancinelli, R. G. Kern, L. J. Rothschild, and C. P. McKay.** 2003. Survival of endospores of *Bacillus subtilis* on spacecraft surfaces under simulated Martian environments: implications for the forward contamination of Mars. Icarus **165:**253–276.
45. **Schuerger, A. C., J. T. Richards, D. A. Newcombe, and K. Venkateswaran.** 2006. Rapid inactivation of seven *Bacillus* spp. under simulated Mars UV irradiation. Icarus **181:**52–62.
46. **van Waasbergen, L. G., D. L. Balkwill, F. H. Crocker, B. N. Bjornstad, and R. V. Miller.** 2000. Genetic diversity among *Arthrobacter* species collected across a heterogeneous series of terrestrial deep-subsurface sediments as determined on the basis of 16S rRNA and *recA* gene sequences. Appl. Environ. Microbiol. **66:**3454–3463.
47. **Venkateswaran, K., S. Chung, J. Allton, and R. Kern.** 2004. Evaluation of various cleaning methods to remove *Bacillus* spores from spacecraft hardware materials. Astrobiology **4:**377–390.
48. **Venkateswaran, K., M. Kempf, F. Chen, M. Satomi, W. Nicholson, and R. Kern.** 2003. *Bacillus nealsonii* sp. nov., isolated from a spacecraft-assembly facility, whose spores are gamma-radiation resistant. Int. J. Syst. Evol. Microbiol. **53:**165–172.
49. **Venkateswaran, K., M. Satomi, S. Chung, R. Kern, R. Koukol, C. Basic, and D. C. White.** 2001. Molecular microbial diversity of a spacecraft assembly facility. Syst. Appl. Microbiol. **24:**311–320.
50. **Welsh, D. T.** 2000. Ecological significance of compatible solute accumulation by microorganisms: from single cells to global climate. FEMS Microbiol. Rev. **24:**263–290.